American Journal of Medical Genetics 62:319–325 (1996)

INTERSTITIAL DELETION OF 11(p11.2p12): A NEWLY DESCRIBED CONTIGUOUS GENE DELETION SYNDROME INVOLVING THE GENE FOR HEREDITARY MULTIPLE EXOSTOSES *(EXT2)*

Lorraine Potocki and Lisa G. Shaffer

Department of Molecular and Human Genetics
Baylor College of Medicine, Houston, Texas

Individuals with deletions of the proximal portion of the short arm of chromosome 11 share many manifestations including mental retardation, biparietal foramina, minor facial anomalies, and multiple cartilaginous exostoses. The finding of multiple exostoses in these patients is remarkable as the disorder hereditary multiple exostoses, which is inherited in an autosomal dominant manner, has recently been mapped by linkage to three regions, including proximal 11p. We report the clinical and molecular findings in an additional patient with an 11(p11.2p12) deletion. Cytogenetic and molecular analysis demonstrated a *de novo*, paternally derived deletion for markers which have been shown to be tightly linked to the 11p locus (*EXT2*). These data support the location of *EXT2* within this region and also provide information regarding the ordering of polymorphic markers on 11p. Deletion 11(p11.2p12) is a rare, yet specific, deletion syndrome involving the *EXT2* locus, a gene for parietal foramina, and a mental retardation locus, and therefore can be classified as a contiguous gene deletion syndrome.

KEY WORDS: multiple exostoses, *EXT2*, parietal foramina, chromosome 11, contiguous gene deletion syndrome.

Address reprint requests to
Lisa G. Shaffer, Ph.D.
Department of Molecular and Human Genetics,
Baylor College of Medicine
One Baylor Plaza, RM 15E, Houston, TX 77030

INTRODUCTION

Contiguous gene deletion syndromes (CGDS) are conditions presumably caused by haploinsufficiency of multiple, functionally unrelated yet physically contiguous loci [Schmickel, 1986; Schinzel, 1988]. Many manifestations in these syndromes may also occur as individual Mendelian traits. For example, supravalvular aortic stenosis may be present as an isolated cardiovascular abnormality or may be found in conjunction with other manifestations of Williams syndrome (deletion 7q11.23) [Olson et al., 1993; Nickerson et al., 1995]. Similarly, the Langer-Giedion syndrome is a contiguous gene syndrome of 8q24 characterized by mental retardation, microcephaly, and multiple exostoses [Bühler et al., 1980; Lüdecke et al., 1991]. The multiple exostoses in patients with Langer-Giedion syndrome are due to deletion of *EXT1* which is a putative tumor suppressor gene which maps to the Langer-Giedion region [Ahn et al., 1995]. Clinical and molecular data support that deletion 11p11.2p12 is also a CGDS which involves the 11p locus associated with multiple exostoses (*EXT2*). Proximal deletions of 11p have been reported previously in only a small number of patients [Francke et al., 1977; Shaffer et al., 1993a; Bartsch et al., 1996]. In addition, McGaughran et al. [1995] report an individual with WAGR (Wilm's tumor, aniridia, genital anomalies, mental retardation), multiple exostoses, and parietal foramina, whose deletion includes the WAGR region (11p13) and extends to the 11p11.2 region. We report the clinical and molecular findings in an additional person with a proximal 11p deletion and discuss the significance of these findings.

CLINICAL REPORT

The patient was referred at age 9 8/12 years for a clinical genetic evaluation following the detection of deletion 11(p11.2p12) on chromosome analysis performed because of craniofacial anomalies and mental retardation. Her prenatal and perinatal course were uncomplicated. Mild global developmental delay and strabismus were noted at 14 months. Chromosomes at this time were apparently normal. She was diagnosed with multiple exostoses and underwent excision of a left humeral exostosis at age 2 1/2 years. Formal developmental testing at 9 years of age showed an IQ of 40-46. On physical exam (Fig. 1) her height, weight, and head circumference were at the 80th, 60th, and 80th centiles, respectively. There was brachycephaly, bilateral parietal foramina of 1-2 cm, epicanthal folds, bilateral ptosis, alternating esotropia, short philtrum, and a downturned upper lip. Multiple bony protuberances were palpated along the ribs, long bones and phalanges. There was no scoliosis. Skeletal radiographs demonstrated multiple exostoses (Fig. 2). An MRI of the brain showed no abnormalities.

Cytogenetic Analysis

Subsequent chromosome analyses were performed at Baylor College of Medicine on peripheral blood lymphocytes from the patient and her parents and demonstrated a *de novo* interstitial deletion in the short arm of one chromosome 11 [46,XX,del(11)(p11.2p12)] (Fig. 3).

Molecular Analysis

Genomic DNA was isolated from the patient and her parents. Analysis of 10 PCR-based dinucleotide repeat polymorphisms was performed. Primer sequences for the Généthon marker *D11S913* (AFM164zf12) were obtained from the Genome Data Base. All other markers were obtained from Research Genetics Map Pairs™. The PCR was performed as previously described [Shaffer et al., 1993b] with the following modifications. The amplification conditions included an initial denaturation at 94°C for 4 minutes followed by 25 cycles of: 94°C denaturation for 1 minute, annealing for 0.5 minutes, and extension at 72°C for 0.5 minutes, followed by a final 7 minute extension at 72°C. The annealing temperature and final concentration of primers were usually 55°C and 0.2 μM, respectively, but these conditions were adjusted individually for each locus examined. Reaction products (8 μl) were mixed with 8 μl of denaturing dye (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) and resolved by electrophoresis through a 7% polyacrylamide sequencing gel containing 5.66 M urea and 32% formamide for 6 hours at 80 watts. Gels were exposed to Kodak film for 0.5 - 12 hours.

Ten PCR-based microsatellite markers mapping to proximal 11p were used to determine the extent and parental origin of the deletion. Seven markers (*D11S935*, *D11S905*, *D11S554*, *D11S1319*, *D11S1313*, *D11S913*, *D11S937*) were fully informative, and 3 were uninformative (*D11S1355*, *D11S903*, *D11S1361*) in this family. The results indicate that this patient failed to inherit a paternal allele at *D11S905*, *D11S554*, and *D11S1319*, while proximal (*D11S1313*) and distal (*D11S935*) markers showed both maternal and paternal alleles (Fig. 4). The patient also inherited both parental alleles for the more centromeric markers *D11S913* and *D11S937* (data not shown).

DISCUSSION

The patient we describe has mental retardation, craniofacial anomalies, multiple exostoses, and a deletion of 11(p11.2p12). Presently, only a few patients are reported with similar deletions [Francke et al., 1977; Shaffer et al., 1993a; Bartsch et al., 1996]. Additionally, Shaffer et al., [1993a] had noted that a patient previously reported [Lorenz et al., 1990] fits the description of their patients, although he did not have a reported chromosome abnormality. Subsequent analysis of this patient has shown a cytogenetic del(11)(p11.2p13), confirmed by molecular studies [Bartsch et al., 1996]. Interestingly, in the present patient and the patients reported previously [Shaffer et al., 1993a], chromosomes were initially also reported as normal. Thus, this small deletion may be relatively difficult to see and may therefore be underascertained.

The characteristic clinical and physical findings of these individuals include developmental delay, mental retardation and minor craniofacial anomalies, including biparietal foramina, large fontanels, brachycephaly, craniosynostosis, epicanthal folds, ptosis, short philtrum, and downturned upper lip (Table I). Malformations of the central nervous system, cardiovascular system, digestive system or genitourinary system have not been reported; however, cryptorchidism and micropenis have been

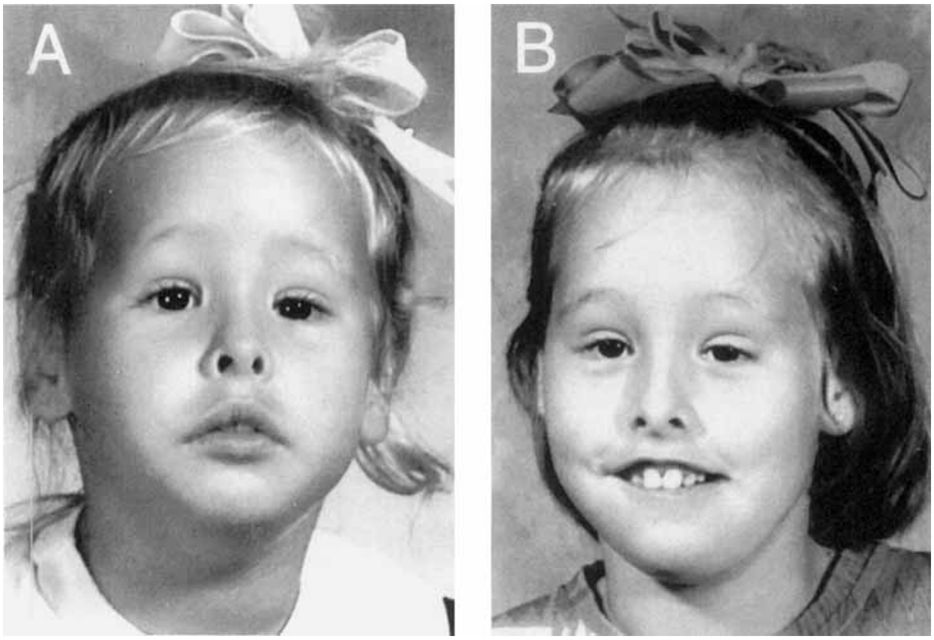

Fig. 1. Patient at four (A) and eight (B) years of age. Note the bilateral epicanthal folds, ptosis, short philtrum, and downturned upper lip.

Fig. 2. Radiograph of the patient's left leg at age 8 years demonstrates an exostosis of the medial aspect of the tibia (arrow).

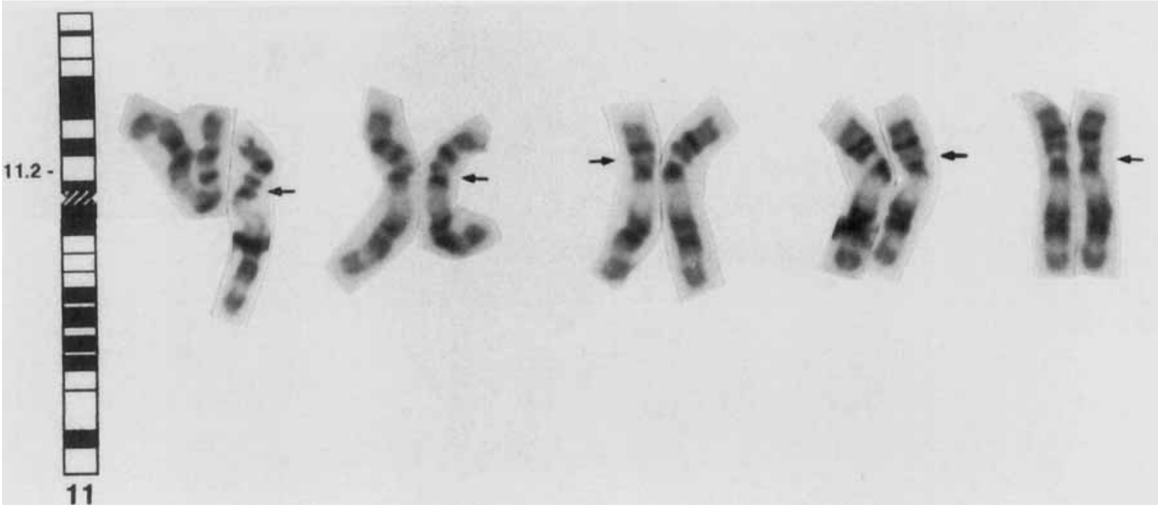

Fig. 3. Idiogram of chromosome 11 and partial karyotype demonstrating the del (11)(p11.2p12) (arrows).

TABLE I. Manifestations of del (11)(p11.2p12)

	Francke et al., 1977	Lorenz et al.,	Shaffer et al., 1993a			McGaughran et al.,	
	Case D	1990	Case 1	Case 2	Case 3	1995	Present Case
Cytogenetic deletion	11(p11.1p1203)	11(p11.12p13)	11(p11.12p12)	11(p11.12p12)	11(p11.12p12)	11(p11.2p14.2)	11(p11.2p12)
Sex	F	M	F	M	M	M	F
Age	5.5 m	2y	4y	16y	6m	26y	9y
Mental retardation	N/A	+	+	+	N/A	+	+
Biparietal foramina	/	+	+	+	-	+	+
Large fontanel	+	+	-	-	-	+	+
Brachycephaly	*	*	+	+	+	/	+
Epicanthal folds	+	-	+	-	+	/	+
Strabismus	/	/	+	+	-	/	+
Short philtrum	+	/	+	+	-	/	+
Downturned upper	/	/	+	+	-	/	+
Micropenis	N/A	+	N/A	+	+	+	N/A
Cryptorchidism	N/A	-	N/A	+	+	+	N/A
Multiple exostoses	/	/	+	+	-	+	+

*Francke et al., 1977 noted dolicocephaly; Lorenz et al., 1990 noted a craniostenotic shape
N/A: not applicable
+: manifestation present
-: manifestation absent
/: not assessed or not reported

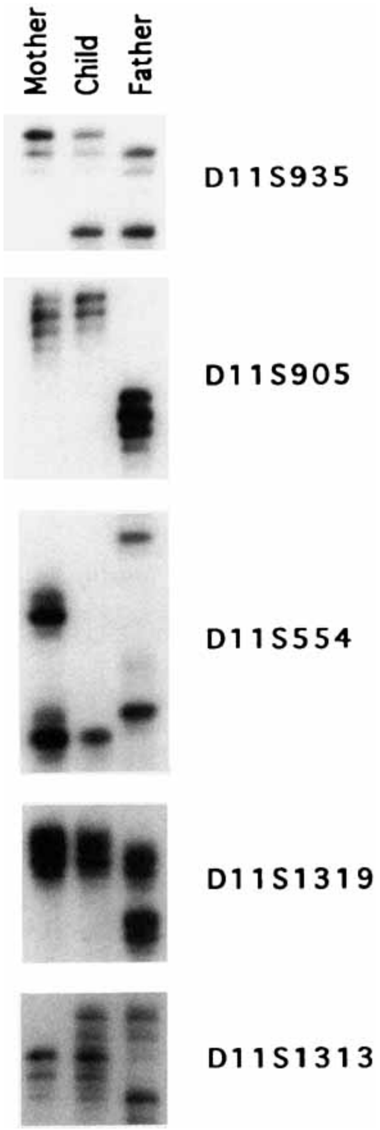

Fig. 4. Molecular analysis using polymorphic dinucleotide markers. The markers are ordered telomeric (distal) to centromeric (proximal) from the top to the bottom of the figure. The child demonstrates inheritance of only one allele (maternal) for markers *D11S905, D11S554*, and *D11S1319*.

noted in males with similar deletions [Shaffer et al., 1993a; Bartsch et al., 1996]. The male patient reported by Shaffer et al. [1993a] also had a seizure disorder.

The most characteristic finding in individuals with proximal 11p deletions is the presence of multiple exostoses. Exostoses are cartilage-capped lesions usually found in the juxtaepiphyseal regions of the long bones but can also be found on the short tubular bones of the hands and feet, tarsal and carpal bones, vertebrae, and sternum [Solomon, 1963; Hennekam, 1991; Schmale et al., 1994]. While exostoses may be present at birth, they often do not develop or do not become apparent until childhood. This is exemplified by the fact that the female patient reported by Shaffer et al. [1993a] did not have exostoses evident by skeletal survey at 2 years; however, exostoses were apparent by skeletal survey performed at age 4 years. The multiple exostoses present in the male patient reported by Shaffer were not initially thought to be associated with the deletion of proximal 11p because there was a history of "bony protuberances" on the paternal side of the family and the deletion was due to abnormal segregation of a maternal insertional translocation. In retrospect, the exostoses were clearly associated with the deletion.

The autosomal dominant disorder, hereditary multiple exostoses, is characterized by the presence of many exostoses but without other associated anomalies. Hereditary multiple exostoses is heterogeneous and maps (by linkage) to 8q (*EXT1*) [Cook et al., 1993], 11p (*EXT2*) [Wu et al., 1994; Wuyts et al., 1995], and 19p (*EXT3*) [Le Merrer et al., 1994]. The *EXT1* gene has been cloned and is thought to be a tumor suppessor gene [Ahn et al., 1995]. The *EXT2* gene has not been cloned. However, linkage data show that the *EXT2* locus maps to the 11p region with the highest lod scores at markers *D11S554, D11S905,* and *D11S903* [Wu et al., 1994; Wuyts et al., 1995]. Recombination events place the gene between markers *D11S1355* and *D11S1361* [Wuyts et al., 1995]. While these markers were uninformative in our family, they are mapped within the deleted interval between markers *D11S905* and *D11S1319* (map order: pter-*D11S935-D11S905-D11S1355-D11S903-D11S1361/D11S554-D11S1319-D11S1313*--qter.) *EXT2* is also thought to function as a tumor suppressor gene as investigators have shown loss of heterozygosity of the *EXT1* and *EXT2* regions in sporadic and exostosis derived chondrosarcomas [Hecht et al., 1995; Raskind et al., 1995]. Although a rare complication of exostoses is the development of chondrosarcoma [Hennekam, 1991; Wicklund et al., 1995], no patient with deletion 11p has yet developed this malignancy. However, follow-up surveillance is indicated in all cases.

The patient described here has a *de novo* deletion of the paternal allele of chromosome 11(p11.2p12). DNA analysis by dinucleotide repeat polymorphisms demonstrated deletion of markers *D11S905, D11S554,* and *D11S1319*. These data support the location of *EXT2* within the 11(p11.2p12) region. The map order of *D11S1313* and *D11S1319* had previously been ambiguous [Gyapay et al., 1994]. Since *D11S1313* is not deleted in our patient, it likely maps more centromeric than *D11S1319*. Since the current case carries a paternal deletion and other patients are deleted for the maternal allele [Shaffer et al., 1993a], *EXT2* does not appear to be subject to imprinting.

The patient reported here and other individuals reported with similar deletions provide phenotypic characterization of a rare, yet specific, deletion syndrome. The current cytogenetic and molecular data in conjunction with published linkage data support that the *EXT2* locus is within the deleted region. The occurrence of multiple exostoses in those hemizygous for the *EXT2* allele suggests that this gene is dosage sensitive or, as suggested by others [Hecht et al., 1995; Raskind et al., 1995], may function as a tumor suppressor gene. Additionally, since patients who have 11p-linked exostoses do not have biparietal foramina or mental retardation [Wu et al., 1994; Wuyts et al., 1995], this deletion most likely represents a true contiguous gene deletion syndrome encompassing the *EXT2* locus.

ACKNOWLEDGEMENTS

The authors thank the family for their participation, Dr. J.R. Lupski for his critical review and helpful discussions of the manuscript, and Bobbye J. Sibley for manuscript preparation.

REFERENCES

Ahn J, Lüdecke H-J, Lindow S, Horton WA, Lee B, Wagner MJ, Horsthemke B, Wells DE (1995): Cloning of the putative tumour suppressor gene for hereditary multiple exostoses (*EXT1*). Nature Genet 11:137-143.

Bartsch O, Wuyts W, Van Hul W, Hecht JT, Meinecke P, Hogue D, Werner W,Zabel B, Hinkel GK, Powell CM, Shaffer LG, Willems PJ (1996): Delineation of a contiguous gene syndrome with multiple exostoses, enlarged parietal foramina, craniofacial dysostosis, and mental retardation, caused by deletions on the short arm of chromosome 11. Am J Hum Genet (in press).

Bühler EM, Bühler UK, Stalder GR, Jani L, Jurik LP (1980): Chromosome deletion and multiple cartilaginous exostoses.Eur J Pediatr 133:163-166.

Cook A, Raskind W, Blanton SH, Pauli RM, Gregg RG, Francomano CA, Puffenberger E, Conrad EU, Schmale G, Schellenberg G, Wijsman E, Hecht JT, Wells D, Wagner MJ (1993): Genetic heterogeneity in families with hereditary multiple exostoses. Am J Hum Genet 53:71-79.

Francke U, George DL, Brown MG, Riccardi VM (1977): Gene dose effect: Intraband mapping of the *LDH A* locus using cells from four individuals with different interstitial deletions of 11p. Cytogenet Cell Genet 19:197-207.

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, Bernardi G, Lathrop M, Weissenbach J (1994): The 1993-94 Généthon human genetic linkage map. Nature Genet 7:246-249.

Hecht JT, Hogue D, Strong LC, Hansen MF, Blanton SH, Wagner M (1995): Hereditary multiple exostosis and chondrosarcoma: Linkage to chromosome 11 and loss of heterozygosity for EXT-linked markers on chromosomes 11 and 8. Am J Hum Genet 56:1125-1131.

Hennekam RCM (1991): Hereditary multiple exostoses. J Med Genet 28:262-266.

LeMerrer M, Legeai-Mallet L, Jeannin PM, Horsthemke B, Schinzel A, Plauchu H, Toutain A, Achard F, Munnich A, Maroteaux P (1994): A gene for hereditary multiple exostoses maps to chromosome 19p. Hum Mol Genet 3:717-722.

Lorenz P, Rupprecht E, Tellkamp H (1990): An unusual type of acrocephalo-syndactyly with bilateral parietooccipital "encephalocele," micropenis, and severe mental retardation. Am J Med Genet 36:265-268.

Lüdecke H-J, Johnson C, Wagner MJ, Wells DE, Turleau C, Tommerup N, Latos-Bielenska A, Sandig K-R, Meinecke P, Zabel B, Horsthemke B (1991): Molecular definition of the shortest region of deletion overlap in the Langer-Giedion syndrome. Am J Hum Genet 49:1197-1206.

McGaughran JM, Ward HB, Evans DGR (1995): WAGR syndrome and multiple exostoses in a patient with del(11)(p11.2p14.2). J Med Genet 32:823-824.

Nickerson E, Greenberg F, Keating MT, McCaskill C, Shaffer LG (1995): Deletions of the elastin gene at 7q11.23 occur in ~90% of patients with Williams syndrome. Am J Hum Genet 56:1156-1161.

Olson TM, Michels VV, Lindor NM, Pastores GM, Weber JL, Schaid DJ, Driscoll DJ,

Feldt RH, Thibodeau SN (1993): Autosomal dominant supravalvular aortic stenosis: Localization to chromosome 7. Hum Mol Genet 2:869-873.

Raskind WH, Conrad EU, Chansky H, Matsushita M (1995): Loss of heterzygosity in chondrosarcomas for markers linked to hereditary multiple exostoses loci on chromosomes 8 and 11. Am J Hum Genet 56:1132-1139.

Schinzel A (1988): Microdeletion syndromes, balanced translocations, and gene mapping. J Med Genet 25:454-462.

Schmale GA, Conrad EU, Raskind WH (1994): The natural history of hereditary multiple exostoses. J Bone Joint Surg 76A:986-992.

Schmickel RD (1986): Contiguous gene syndromes: A component of recognizable syndromes. J Pediatr 109:231-241.

Shaffer LG, Hecht JT, Ledbetter DH, Greenberg F (1993a): Familial interstitial deletion 11(p11.12p12) associated with parietal foramina, brachymicrocephaly, and mental retardation. Am J Med Genet 45:581-583.

Shaffer LG, Overhauser J, Jackson LG, Ledbetter DH (1993b): Genetic syndromes and uniparental disomy: A study of 16 cases of Brachmann-de Lange syndrome. Am J Med Genet 47:383-386.

Solomon L (1963): Hereditary multiple exostoses. J Bone Joint Surg 45B:292-304.

Wicklund CL, Pauli RM, Johnston D, Hecht JT (1995): Natural history study of hereditary multiple exostoses. Am J Med Genet 55:43-46.

Wu Y-Q, Heutink P, de Vries BBA, Sandkuijl LA, van den Ouweland AMW, Niermeijer MF, Galjaard H, Reyniers E, Willems PJ, Halley DJJ (1994): Assignment of a second locus for multiple exostoses to the pericentromeric region of chromosome 11. Hum Mol Genet 3:167-171.

Wuyts W, Ramlakhan S, Van Hul W, Hecht J, van den Ouweland AMW, Raskind WH, Hofstede FC, Reyniers E, Wells DE, de Vries B, Conrad EU, Hill A, Zalatayev D, Weissenbach J, Wagner MJ, Bakker E, Halley DJJ, Willems PJ (1995): Refinement of the multiple exostoses locus (EXT2) to a 3-cM interval on chromosome 11. Am J Hum Genet 57:382-387.